Research Project Proposal

On

Studies on neuropeptide Y in the treatment of breast cancer

To be submitted

to

Research and Recognition Committee

of

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In

Pharmaceutical Sciences

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1. Introduction

Most common types of cancers in women are lung, cervix, colorectal, thyroid and breast cancer(BC). According to WHO, 1 in 8 women is diagnosed with BC and 6,27,000 women died from BC in 2018. In India, BC incidence is higher in females in urban areas as compared to the rural. Tumor node metastatic (TNM) staging system is classified as operable (stage T1- ≤2cm), large operable (stage T2- 2-5cm), locally advanced (stage T3- >5cm) and metastatic (stage T4- spread) BC. It is diagnosed by blood tests, breast imagining (mammography, MRI), biopsy, chest X-ray and bone scan. Systemic therapy used in BC includes chemotherapy with taxanes like paclitaxel or docetaxel, adjuvant therapy like trastuzumab, radiotherapy or tamoxifen with surgical intervention. For severe stages, lumpectomy or mastectomy or breast reconstruction is preferred over conventional treatment options. (1,2)

Neuropeptide Y (NPY), a polypeptide of 36 amino acid sequence (Tyr-Pro-Ser-Lys-Pro-Asp-Asn-Pro-Gly-Glu-Asp-Ala-Pro-Ala-Glu-Asp-Met-Ala-Arg-Tyr-Tyr-Ser-Ala-Leu-Arg-His-Tyr-Ile-Asn-Leu-Ile-Thr-Arg-Gln-Arg-Tyr-NH_2) with molecular weight 4253.7 g/mol and starting and ending with tyrosine moiety mostly present in the brain. NPY follows flip-flop pharmacokinetics, i.e., slow absorption and fast elimination possessing half-life of 2 min to 2 days with low oral bioavailability (≤ 1%) hence administered via subcutaneous or intramuscular route. The synthetic procedure for preparation of NPY_{3-36} are shown in figure 1. It is used for various physiological processes for management and treatment of various ailments related to central nervous system, cardiovascular system, respiratory system, gastro-intestinal system and endocrinal system. NPY is thermoliable, stable below -20 ^{o}C and results in rapid enzymatic degradation and inactivation of the peptide. It undergoes polymorphism by changing leucin to proline elevating cholesterol, alcohol consumption, damaging cardiovascular and metabolic system. Hence, stabilization of NPY is a major challenge which can be improved by using different carrier systems a like lipids, polymers, saccharides, etc. NPY targets tumor cells by acting on endosomes releases cytolysin to break disulfide linkers in the cytoplasm which regulates tumor growth by induction or inhibition of apoptosis. A high density of human Y_1 receptor expressed in metastasis and breast cancer cells whereas selective Y_2 receptor analogs are effective on breast and pancreatic cancers by an increase of calcium concentration intracellularly and

reduction of forskolin-induced cAMP accumulation. NPY (mostly Y_1 receptor) is overexpressed in BC and used for diagnosis as well as tumor-specific targeted therapy using NPY carrier systems. (3,4)

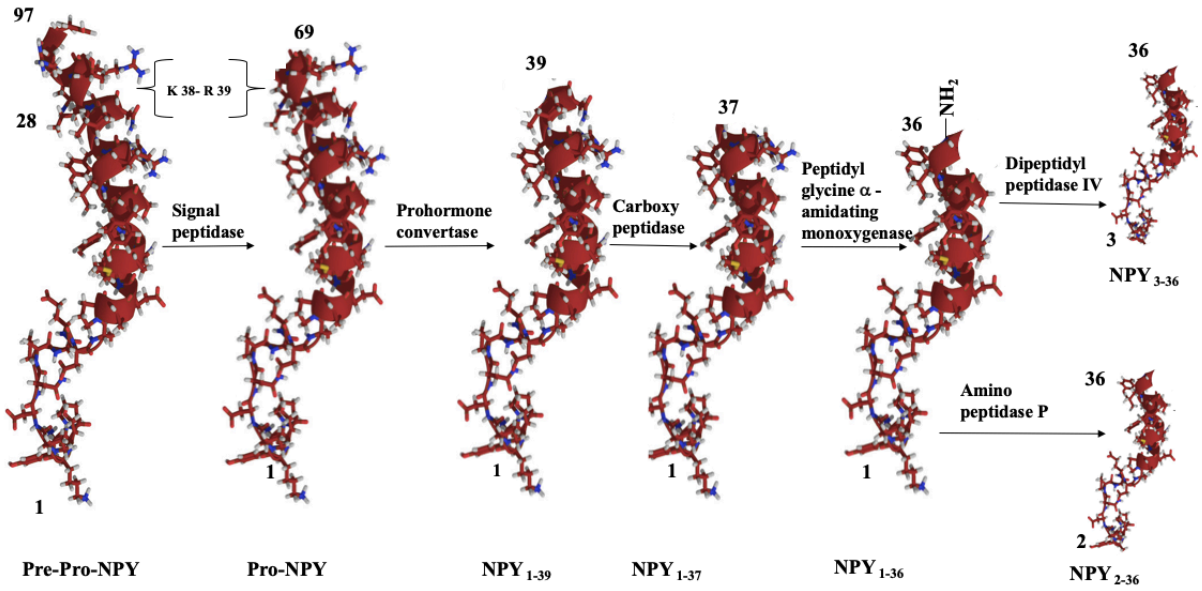

Figure 1. Synthesis of NPY_{3-36}

Lipids are widely used in drug and gene delivery in the form of liposomes and lipoplexes. It used cationic, anionic, neutral, or a mixture of lipids with DNA. Lipids help to stabilize the peptide and show better delivery and efficacy. The lipoplex interaction with peptide affects internalization, cell association, transfection efficacy and nuclear accumulation through various peptide: DNA w/w ratios with lipid complex. Lipids on combination with peptide: DNA stabilizes peptide and shows better therapeutic efficacy. NPY helps in angiogenesis by triggering hypoxia whereas DNA interfere with nucleus and disrupts the cell. Anionic lipids like cetrimide, palmitic acid, stearic acid, etc. are used to for DNA complex affecting nuclear accumulation and increases transfection efficacy. (5-7)

Interaction of polymer mostly cationic with DNA form polyplexes helps to cross blood brain barrier providing stability and used as an alternative therapy to viral vectors and drug delivery improving efficacy. Nano-polyplexes are formulated to deliver intracellular acting peptides using anionic polymers like poly(propylacrylic acid) to enhance peptide retention and uptake. DNA fuses with endoplasmic reticulum leading

to lysosomal disruption and membrane destabilization whereas NPY activates MAPK(mitogen-activated protein kinase) pathway and regulates cell proliferation. Polyplexes improves the water solubility of NPY by modifying particle surface and protects the peptide from degradation serving as tumor-specific cell penetrating peptide. Polymers like poly (propylacrylic acid) are used to increase solubility and enhance peptide retention and uptake. (8-12)

Aggregated nanoparticles form nanocluster cross-linked with peptide increasing the area for entrapment with lipid by layer to provide stability and better release. Mostly desolvation method is used for intracellular peptide delivery. Thiolated peptide templates are used to prepare semiconducting and magnetic nanometric materials like stabilized nanoclusters as next-generation application to nanomedicine and theronostics. Various approached to prepare nanoclusters are chemical etching, photoreduction, electrochemical or sonochemical synthesis, microwave-assisted synthesis and chemical reduction. Peptide is protected by formation of nanocluster and stabilized by lipid layering. (13-15)

Polysaccharides nanoparticles are sugar-based biodegradable nanocarriers used to bind overexpressed receptors in tumor cells. Due to hydrophobic interactions of polysaccharides with peptide, it improves solubility, stability and release pattern. It is prepared by surface modification, physical or chemical crosslinking and amphiphilic derivate synthesis. Formation of inclusion complex promotes endosomal peptide release with targeted delivery. Peptide complex was found to treat breast cancer by breakdown of protein and supressing malignant cells. β-cyclodextrin entraps hydrophobic agents and acts as superior carrier for peptide delivery. On lipid coating, peptide gets stabilized whereas polysaccharides help in NPY delivery for targeted action. Various polymers like cyclodextrin, chitosan, pullulan, dextran, etc. can be used for peptide delivery for breast cancer. (16-19)

Layer-by-layer (LBL) coating with NPY on all the prepared formulation helps to achieve overexpression of the peptide and target Y_1 receptor to treat BC. LBL is used to deliver DNA, proteins, viruses, polypeptides, etc. in the form of film through hydrogen bonding or electrostatic or hydrophobic forces. It is prepared by various

methods like dip, spin and spray coating. It is simple, robust, reproducible and assembly technique. (20)

2. Rationale

- **NPY**

  NPY, an unstable easily degradable peptide, is a choice of interest for various treatment. The overexpression of the NPY when administered intravenously, target Y_1 receptor to treat BC with potential for better patient efficacy and safety.

- **Lipoplexes and polyplexes**

  Lipid- and polymer-based peptide complex improves peptide solubility and stability for treatment of breast cancer. It is widely applicable for gene delivery with enhanced efficacy and stability.

- **Peptide nanocluster**

  Nanoclusters act a potential carrier for NPY due to increased surface area and better binding capacity. The formulation can be altered to different routes of administration and enhances stability of the peptide. The clusters formed by direct process helps the peptide to remain intact as it may degrade due to its thermolabile property.

- **Inclusion complex**

  Inclusion complex formation with peptide and carbohydrates stabilizes the peptide and helps to improve solubility and efficacy to provide targeted delivery. It helps in controlled-release of the peptide due to electrostatic bond formed by surface charges interaction of carbohydrates and overexpressed peptide.

- **Layer-by-layer (LBL)**

  LBL technique protects the NPY from degradation due to layer deposition with improved and controlled peptide release. Accumulation by LBL improves the nanoparticle carriers stability and systemic circulation.

3. Objective

- Stabilization of NPY using complexation, plexes, clusters and LBL
- Formulation and characterization of NPY lipoplexes
- Formulation and characterization of NPY polyplexes
- Formulation and characterization of NPY peptide clusters
- Formulation and characterization of NPY inclusion complex

4. Approaches to the issues

NPY is used in various disease treatment but its stability restricts its usage. Lipoplexes, polyplexes, nanoclusters, polysaccharide nanoparticles and inclusion complex with LBL coating represents an emerging novel approaches for peptide delivery with various advantages like improved solubility and stability of the peptide as it is a major concern for peptide delivery. The dose frequency and dosage is reduced as compared to the available tablets increasing bioavailability due to direct peptide delivery. The prepared formulations will be evaluated for various evaluation parameters like morphological studies, particle size, zeta potential, solubility studies, entrapment efficiency, *in-vitro* release study, NMR, cell line study and stability study.

5. Literature

Literature search

Scala C. et al. proposed gene delivery is possible with polymer-based engineered system showing potential efficacy and minimizing side effects. It acts as a novel polymer-based approach for *in vivo* transfection in postnatal brain. (21)

Shende P. et al. described complex forms of lipids and polymers used as an alternative to viral gene carriers. They act by interacting with cell surface causing breakdown of nucleic acid in cytosol. They are prepared by various methods like direct mixing, thin film hydration, using microfluids, etc. (12)

Li Q., et al. synthesized peptide protected gold nanoclusters by spontaneous process to show antimicrobial activity. They prepared a chemically-triggered delivery system using vancomycin with peptide gold nanoclusters. (22)

Rica R., et al. designed a helical peptide used to form caged gold nanocluster. Peptide templates are used to prepare semiconducting and magnetic nanometric materials like nanoclusters as next-generation application to nanomedicine and theronostics. (23)

Hanieh H, et al. immobilized peptide on the surface helping target bacteria to bind from the contaminated sample. *In-situ* gold nanoclusters are prepared on glass slide to act as biosensor being more sensitive and faster analysis using MALDI spectroscopy. (24)

Marqus S, et al. evaluated peptides for treatment of cancer. As peptides show high target specificity, selectivity and low toxicity rather than proteins and antibodies, PEGylation occurs is another approach to increase the half-life of peptides. (25)

Li J., et al. prepared doxorubicin albumin encapsulated nanoparticles with modified NPY ligand conjugation meditate breast cancer targeted delivery by overexpressing NPY to Y_1 receptors with minimal normal cell damage. (26)

Liu Z., et al. prepared polysaccharide-based nanoparticulate systems by covalent crosslinking, ionic crosslinking, polyelectrolyte complexation and self-assembly methods. Polymers like chitosan, cyclodextrin, etc. shows biocompatibility with peptide interface. (17)

Patent search

Title	**Applicant/innovator**	**Filling date**
Cancer-targeting peptides and uses thereof in cancer therapy	Han-Chung W., Chien-Yu C. (Current assignee: Academia Sinica)	19/02/2009
Neuropeptide Y ligands	Ling A., Tompkins E., Hong Y., Gregor V. (Current assignee: Alanex Corp.)	23/08/1996
Lipoplexes	Yoshiaki O., Yu T., Ayako A., Lin X., Madon T.	07/10/2011
Method for preparing homogenous liposomes and lipoplexes	Garidel P., Hans H., Denkinger N. (Current assignee: Boehringer Ingelheim Pharma GmbH and Co KG)	05/09/2003
Polyplexes of hydrophobically-modified siRNA for delivery of siRNA	Vetro JA. (Current assignee: University of Nebraska)	01/11/2011
Nanocluster capped mesoporous nanoparticles, methods of making and use	Shahad A., Mohamed A., Niveen K.	03/10/2016
Polypeptide-modified gold nanocluster and preparation method thereof	Wang X., Qu J., Wang Y., Huang F., He H	02/06/2016

Biosynthetic gene cluster for the production of peptide/protein analogues	Jorn P., Gurgui C., Michael F., Agustinus R., Maximilian J.	05/09/2011
Use of breast cancer associated membrane proteins (bcmp) for treatment, prophylaxis and diagnosis of breast cancer	Robert Simon Oxford Glycosciences Boyd (Uk) Ltd.	25/02/2000
Layered Nanoparticles for Sustained Release of Small Molecules	Louisiana State University and Agricultural and Mechanical College Louisiana Tech University Research Foundation	31/03/2006

6. Work Plan and Research Methodology

- Literature and patent survey
- Listing and procurement of chemicals

a. Methods

- Characterization of NPY and excipients
- LBL coating to enhance stability
- Formulation and characterization of lipoplexes
- Formulation and characterization of polyplexes
- Formulation and characterization of peptide clusters
- Formulation and characterization of inclusion complex

b. Stability study of NPY carriers

c. Characterization for lipoplexes, polyplexes, peptide clusters and inclusion complex

- Morphology characteristics
- Particle size and zeta potential
- FTIR
- DSC
- Solubility studies
- Entrapment efficiency

- *In vitro* release study
- NMR

d. Cell line studies

e. Publication

f. Thesis submission

7. Work Timeline

Sr. No.	Work or activity	Time required
1	Literature Search and review, procurement of API and excipients	2 months
2	Characterization of API and excipients	2 months
3	Formulation and analytical method development for lipoplexes	3 months
4	Formulation and analytical method development for polyplexes	3 months
5	Formulation and analytical method development for peptide cluster	3 months
6	Formulation and analytical method development for peptide inclusion complex nanoparticles	3 months
7	Characterization of lipoplexes, polyplexes, peptide cluster, polysaccharide nanoparticles and inclusion complex	6 months
8	Stability studies and cell line studies of prepared formulations	3 months
9	Data compilation and publications	3 months
10	Thesis submission	2 months
Total		**30 months**

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